MOUSE LUNG ADENOCARCINOMA CELL LINES REVEAL PRL2C2 AS A NOVEL LUNG TUMOUR PROMOTER

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Background Carcinogen-inflicted human cancers, including lung tumours harbour thousands of mutations per genome, most of which are unknown (Garraway, LA et al., Cell 2013;153:17–37).

Aim To develop a faithful mouse model of human tobacco carcinogen-induced lung adenocarcinoma suitable for the identification of novel oncogenic genes and pathways.

Methods We repeatedly managed to obtain several murine lung adenocarcinoma cell lines (MLA) by chronically exposing various mouse strains to different tobacco carcinogens. MLA were characterised for cancer stemness and oncogenes, as well as global gene expression.

Results To date, 12 MLA cell lines have been derived from WT and transgenic mice on the FVB, Balb/c, and C57BL/6 Strains by means of urethane or diethylnitrosamine exposure. All MLA were immortal, phenotypically stable, and indefinitely passaged in vitro over a period of over 18 months and/or 60 passages. In addition, all cell lines were oncogenic, transplantable, metastatic, and uniformly lethal in vivo. Interestingly, MLA displayed Kras mutations in codon 61, mono- or bi-allelic Trp53 loss, and expression of lung cancer stemness factors Itgb3 and Lgr6, in amazing similarity to human lung cancers. Microarray revealed that all MLA cell lines heavily overexpressed Prl2c2, encoding proliferin, in comparison to the native lungs. Prl2c2 silencing diminished MLA proliferation and stemness, to a degree comparable with Itgb3 interference.

Conclusions MLA are faithful models of human lung adenocarcinoma that led to the discovery of Prl2c2 as a candidate lung tumour promoter.

Funding European Research Council Starting Independent Investigator Grant #260524. Respire 2 European Respiratory Society Fellowship, European Respiratory Society Short Term Research Fellowship.

OSTEOPONTIN AS AN AIRWAY EPITHELIAL TUMOUR PROMOTER

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Osteopontin (secreted phosphoprotein 1; SPP1) expression has been identified in human lung cancer and has been linked with enhanced lung tumour progression. To examine its functional role, we induced lung tumours by repetitive urethane or MCA/BHT lung carcinogens in C57BL/6 mice lacking both (Spp1-/-), one (Spp1 +/−), or no (Spp1+/+) copy of the endogenous Spp1 gene. Primary end-points were lung tumour number and size; secondary end-points were SPP1 expression, epithelial cell survival, carcinoma-induced inflammation, and angiogenesis. Data are presented as mean ± SD.

Compared with Spp1+/+ mice (n = 22), Spp1−/− mice (n = 25) developed dramatically fewer and significantly smaller lung tumours in response to urethane, while Spp1+/− mice (n = 12) behaved similar to Spp1−/− mice (number/diameter of lung tumours in Spp1+/+ vs. Spp1+/−, and Spp1−/− mice, respectively: 16.1 ± 12.7/1.2 ± 0.3 mm, 2.4 ± 2.3/0.9 ± 0.2 mm, and 1.3 ± 1.6/0.7 ± 0.2 mm; P < 0.05 for comparison of Spp1+/+ with Spp1−/− and Spp1+/− mice). Spp1−/− mice were also protected from two-hit MCA/BHT-oncogenesis compared with Spp1+/+ controls. Spp1−/− mice displayed decreased epithelial cell survival and reduced numbers of airspace macrophages early after urethane, and enhanced tumour cell apoptosis and limited tumour angiogenesis at late stages of lung tumour progression. SPP1 was expressed in the naïve lung by non-ciliated airway epithelial cells and alveolar macrophages and was significantly up-regulated during multi-stage lung carcinogenesis.

Our data indicate that SPP1 is functionally involved in airway epithelial carcinogenesis and may present a target for lung cancer treatment and prevention.

THE ROLE OF LRIG1-DEPENDENT EGFR SIGNALLING IN AIRWAY HOMEOSTASIS AND SQUAMOUS CELL LUNG CANCER DEVELOPMENT


Background Aberrations of EGFR signalling drive cancer development. In squamous cell lung cancer (SqCLC), EGFR is overexpressed. LRIG1 is a negative regulator of EGFR and patient pre-invasive SqCLC samples show LRIG1 loss, suggesting involvement in early disease pathogenesis. In skin and gut homeostasis, LRIG1 regulates stem cells. In the upper airway, basal cells act as stem cells and are the putative origin of SqCLC. We hypothesise LRIG1 has a key role in airway carcinogenesis and its loss promotes pre-invasiveSqCLC development.

Methods Lrig1 EGFP-Ires-CreERT2 mice were used to delineate airway LRIG1 expression. Flow sorted Lrig1-positive and -negative murine basal cells were used in 2D and 3D colony-forming, spheroid and proliferation assays. A murine SqCLC model was set up through application of N-Nitrosotris-(2-chloroethyl)urea (NTCU). Pre-invasive lesions and tumour development were compared between wild-type (WT), heterozygous and LRIG1-knockout (KO) animals. Human basal cells obtained from bronchoscopy were sorted according to LRIG1 expression and used in organotypic models.

Results Lrig1 is expressed by 40% of airway basal cells. Lrig1-expressing murine basal cells exhibit increased colony-forming capacity (p = 0.0286), spheroid formation (p = 0.0043) and...